First Experimental Demonstration of the Multipotential Carcinogenic Effects of Aspartame Administered in the Feed to Sprague-Dawley Rats

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The Cesare Maltoni Cancer Research Center of the European Ramazzini Foundation has conducted a long-term bioassay on aspartame (APM), a widely used artificial sweetener. APM was administered with feed to 8-week-old Sprague-Dawley rats (100–150/sex/group), at concentrations of 100,000, 50,000, 10,000, 2,000, 400, 80, or 0 ppm. The treatment lasted until natural death, at which time all deceased animals underwent complete necropsy. Histopathologic evaluation of all pathologic lesions and of all organs and tissues collected was routinely performed on each animal of all experimental groups. The results of the study show for the first time that APM, in our experimental conditions, causes *a*) an increased incidence of malignant-tumor–bearing animals with a positive significant trend in males ($p \le 0.05$) and in females ($p \le 0.01$), in particular those females treated at 50,000 ppm ($p \le 0.01$); *b*) an increase in lymphomas and leukemias with a positive significant trend in both males ($p \le 0.05$) and females ($p \le 0.01$), in particular in females treated at doses of 100,000 ($p \le 0.01$), 50,000 ($p \le 0.01$), 10,000 ($p \le 0.05$), 2,000 ($p \le 0.05$), or 400 ppm ($p \le 0.01$); *c*) a statistically significant increased incidence, with a positive significant trend ($p \le 0.01$), of transitional cell carcinomas of the renal pelvis and ureter and their precursors (dysplasias) in females treated at 100,000 ($p \le 0.01$), 50,000 ($p \le 0.01$), 10,000 ($p \le 0.01$), 2,000 ($p \le 0.05$), or 400 ppm ($p \le 0.05$); and *d*) an increased incidence of malignant schwannomas of peripheral nerves with a positive trend ($p \le 0.05$) in males. The results of this mega-experiment indicate that APM is a multipotential carcinogenic agent, even at a daily dose of 20 mg/kg body weight, much less than the current acceptable daily intake. On the basis of these results, a reevaluation of the present guidelines on the use and consumption of APM is urgent and cannot be delayed. *Key words:* **artificial sweetener, aspartame, carcinogenicity, lymphomas, malignant schwannomas, rats, renal pelvis carcinomas.** *Environ Health Perspect* **114:379–385 (2006). doi:10.1289/ehp.8711 available via *http://dx.doi.org/* [Online 17 November 2005]**

Consumers are increasingly concerned about the quality and safety of many products present in the diet of industrialized countries, in particular, the use of artificial sweeteners, flavorings, colorings, preservatives, and dietary supplements. General apprehension also exists regarding the possible long-term health effects of the raw materials and technologies used for the packaging, sterilization, and distribution of foods. Of particular concern are the potential carcinogenic effects of these products and processes.

The experimental and epidemiologic data currently available to evaluate the above carcinogenic risks are insufficient and often unreliable because of the inadequate planning and conduct of previous experiments. This inadequacy, combined with the general limited knowledge about the safety and potential carcinogenic effects of substances widely present in the industrialized diet, motivated the design of an integrated project of mega-experiments in 1985 at the Cesare Maltoni Cancer Research Center (CMCRC) of the European Ramazzini Foundation (ERF). The products studied are reported in Table 1. The products and agents we selected for this project were those for which committee debate and opinions had often acted as surrogates for good laboratory work. At present, over the course of the project, 32 long-term bioassays have been performed using > 25,000 rodents. Studies have evaluated the carcinogenicity of 12 different products, including the artificial sweetener aspartame (APM).

In this article we present the results of the mega-experiment on the carcinogenicity of APM in which the sweetener was administered in feed to Sprague-Dawley rats for the life span.

APM, the methyl ester of the dipeptide L-α-aspartyl-L-phenylalanine ($C_{14}H_{18}N_2O_5$), is a widely used artificial sweetener with a molecular weight of 294.3. Under particular conditions (extreme pH, high temperature, lengthy storage times), APM may be contaminated by the diketopiperazine (DKP) cycloaspartylphenylalanine (Butchko et al. 2002a).

For more than 30 years, APM has been widely used as a food additive because of its very strong, sweet taste. The sweetening power of APM is estimated to be 200 times that of sucrose, whereas saccharin and cyclamate are 300 and 30 times sweeter, respectively (Mazur 1984).

Initial commercial approval of APM in the United States was granted by the Food and Drug Administration (FDA 1974). The FDA later approved the limited use of APM in solid foods in 1981 and extended this authorization to soft drinks in 1983. APM was eventually approved as a general sweetener in 1996 (FDA 1981, 1983, 1996). In the European Union, the safe use of APM was authorized in 1994 (EC Directive 1994).

After saccharin, APM is the second most used artificial sweetener in the world. It is estimated that > 8,000 tons of APM are consumed each year in the United States (Hazardous Substances Data Bank 2005). In terms of world consumption, APM represents 62% of the value of the intense sweetener market (Fry 1999).

APM is found in > 6,000 products, including carbonated and powdered soft drinks, hot chocolate, chewing gum, candy, desserts, yogurt, tabletop sweeteners, and some pharmaceutical products, such as vitamins and sugar-free cough drops, and is estimated by the Aspartame Information Center (2005) to be consumed by > 200 million people worldwide.

Through dietary surveys performed in the United States among APM consumers during the period 1984–1992, the average APM daily intake in the general population has been shown to range from 2 to 3 mg/kg body weight (bw). Consumption by children 2–5 years of age and by females of childbearing age in these surveys ranged from about 2.5 to 5 mg/kg bw/day (Butchko et al. 2002b). APM intake was also monitored in several other regions, including seven European countries. Although survey methodologies may have differed, the APM intake was remarkably consistent across studies and was well below the acceptable daily intake (ADI) both in the United States (50 mg/kg bw) and in Europe (40 mg/kg bw) (Butchko et al. 2002b).

Investigations into the metabolism of APM have shown that, in rodents, nonhuman

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